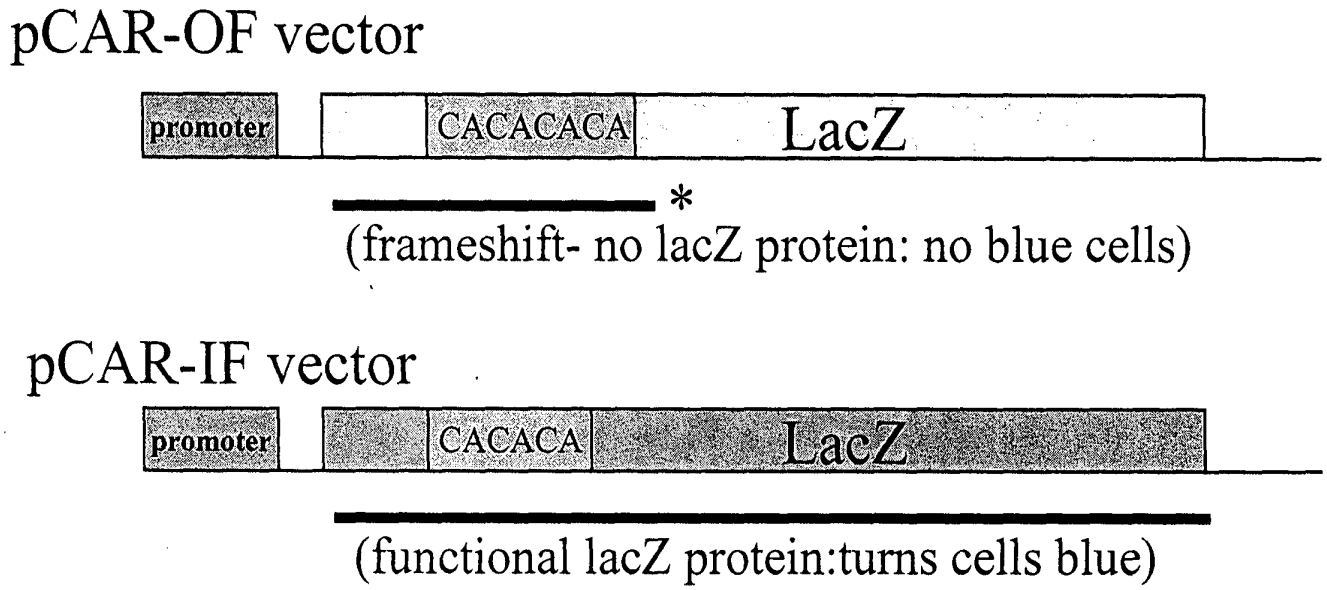


Figure 1. Schematic diagram of pCAR vectors. Each vector contains a polyCA repeat at the N-terminus. The pCAR-OF vector contains an out-of-frame β-gal and therefore does not produce LacZ activity.

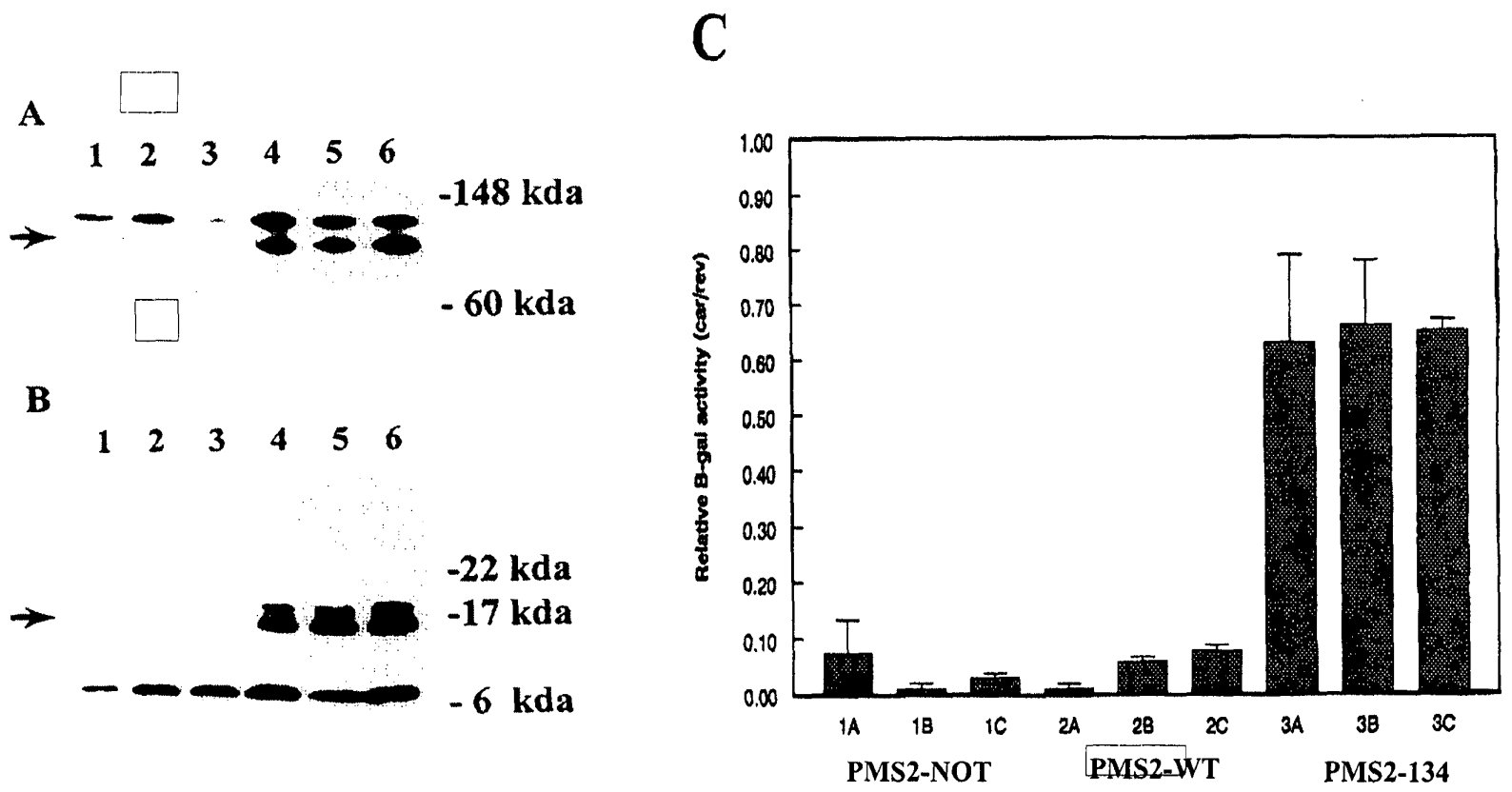


Figure 2: SH cells were transfected with pCAR-OF plus PMS2-NOT, PMS2-WT or PMS2-134. (A) Western blot of cells transfected with PMS2-NOT or PMS2-WT. Lanes 1-3 are cells transfected with PMS2-NOT + pCAR-OF, Lanes 4-6 are cells transfected with PMS2-WT + pCAR-OF. (B) Western blot of cells transfected with PMS2-NOT or PMS2-134 Lanes 1-3 are cells transfected with PMS2-NOT + pCAR-OF, Lanes 4-6 are cells transfected with PMS2-134 + pCAR-OF . (C) β-gal activity of cells expressing the pCAR reporter plasmids and the PMS2 effector plasmids indicates expression of functional LacZ gene (due to in vivo genetic alterations) from pCAR-OF vector due to defective MMR occurs only in cells expressing the dominant negative PMS2-134 polypeptide.

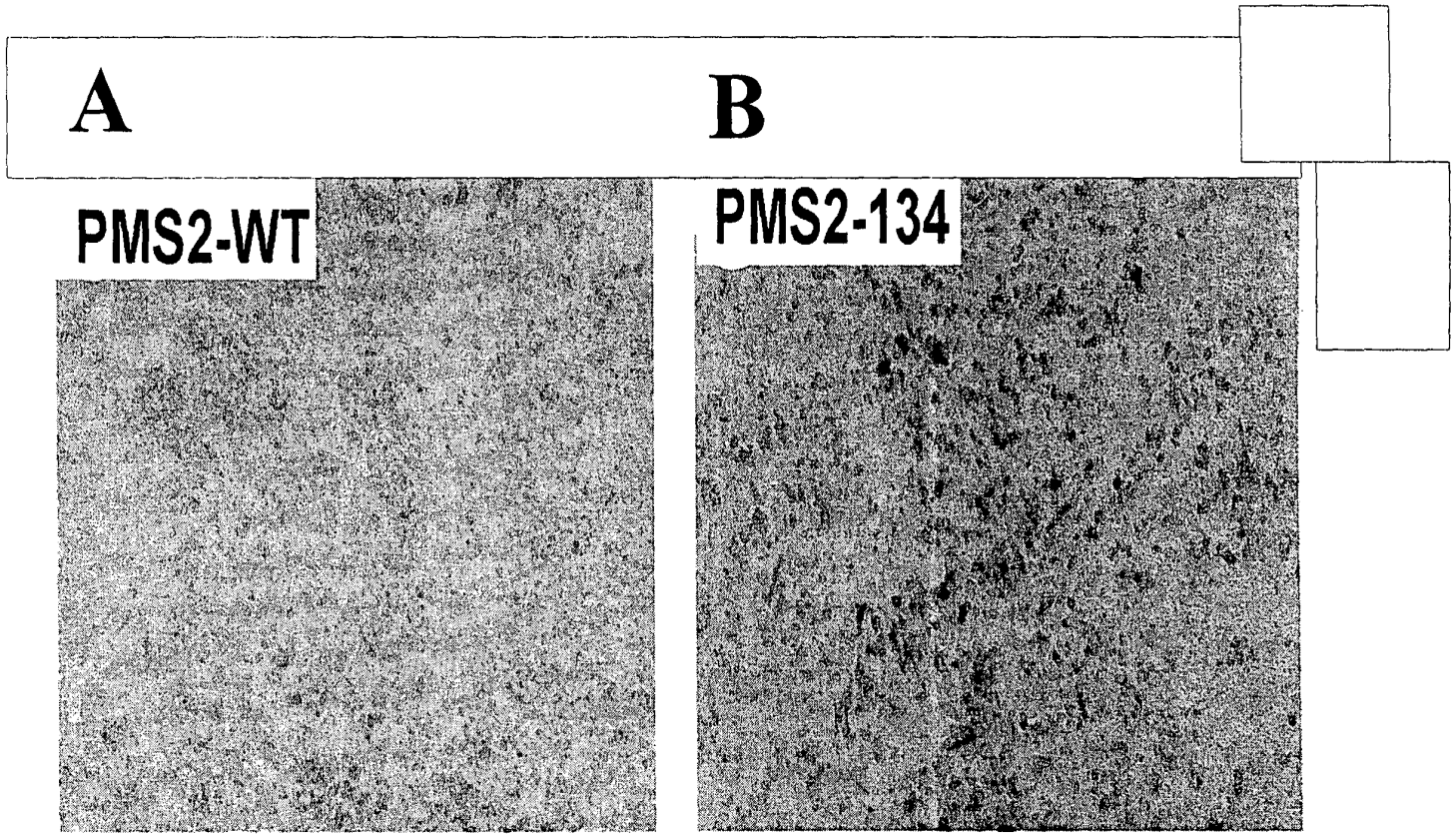
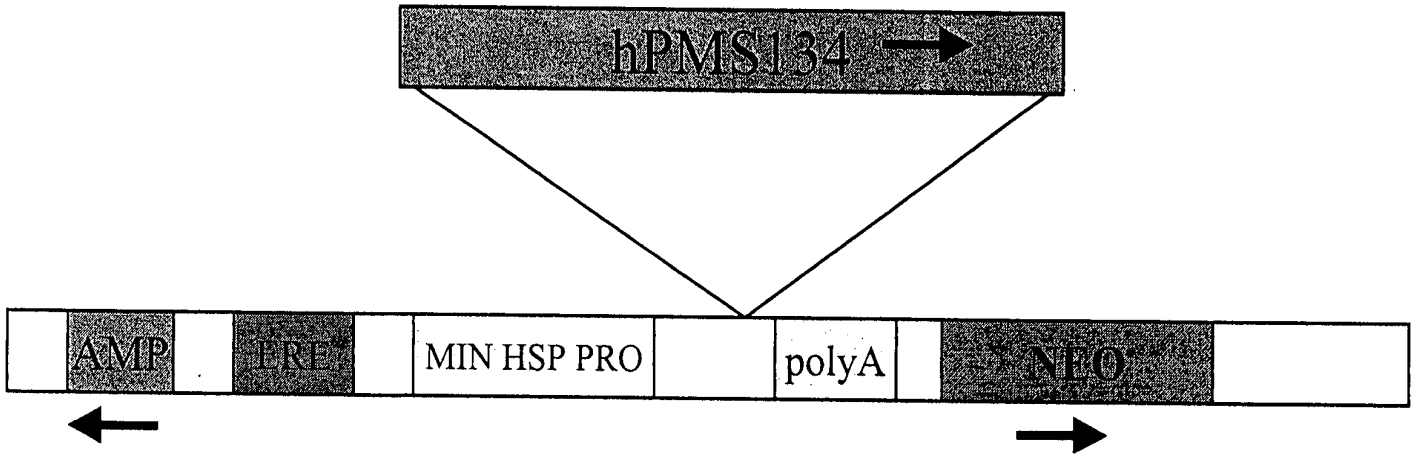


Figure 3. SH cells expressing pCAR-OF and PMS2-WT (A) or pCAR-OF and PMS2-134 (B). SH cells expressing the PMS2-134 gene have defective MMR and alter the pCAR-OF gene structure in vivo to produce an in-frame β-gal gene and a functional LacZ enzyme



AMP= ampicillin resistance gene
ERE= ecdysone response elements
MIN HSP PRO= minimal heat shock promoter
hPMS134= dominant negative MMR allele
polyA= BGH polyadenylation signal
NEO= neomycin phosphotrasferase gene

Figure 4: Schematic diagram of the pINDPMS134 steroid inducible dominant negative MMR gene expression vector.

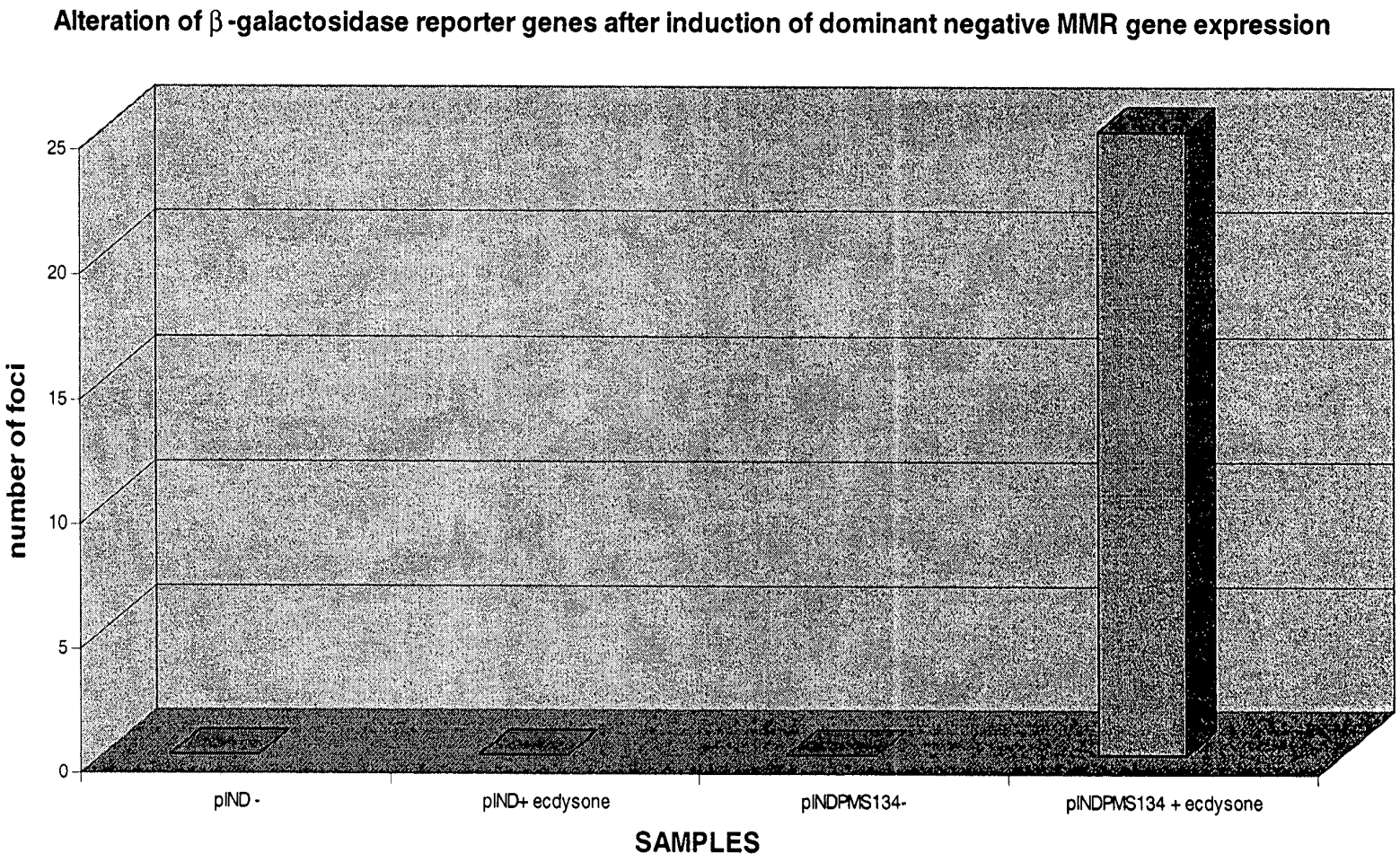


Figure 5. Inducible regulation of dominant negative MMR genes.

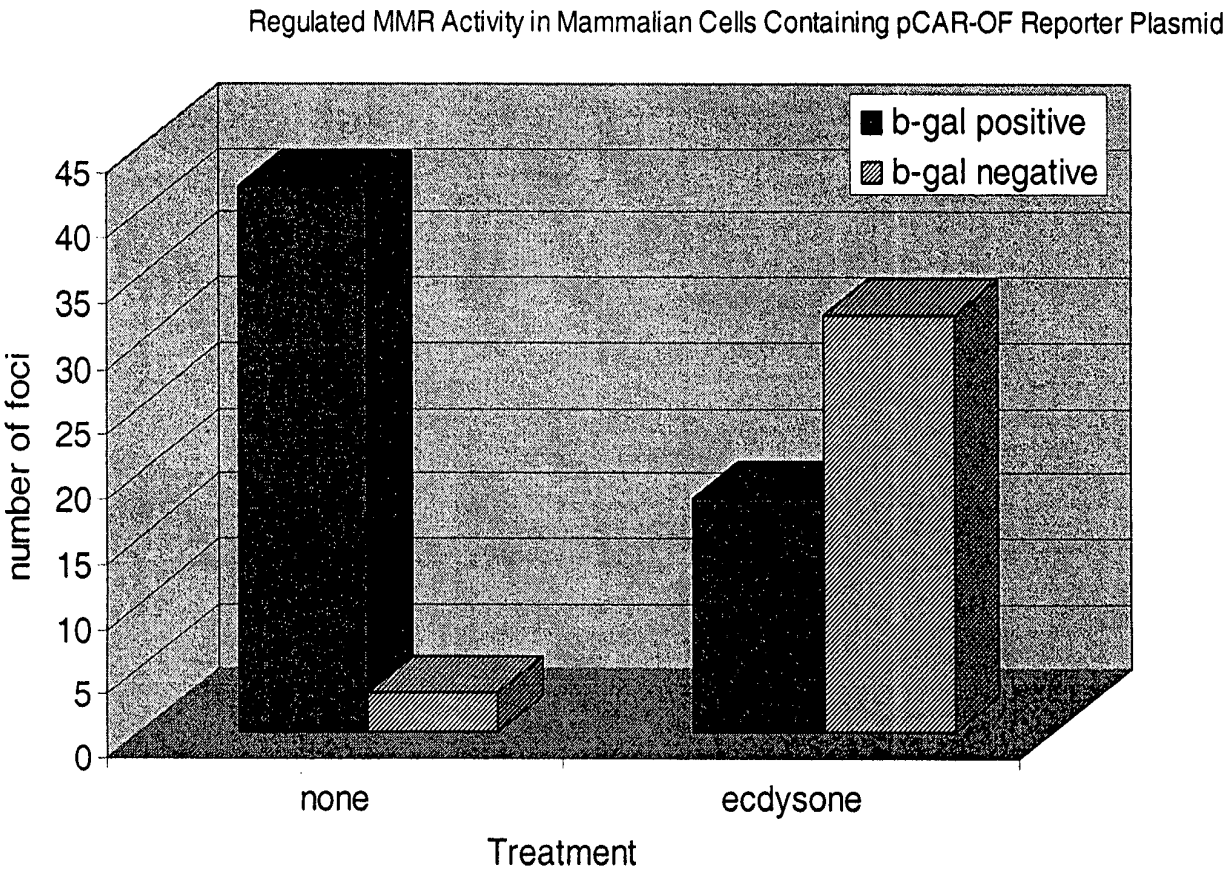


Figure 6. Inducible regulation of dominant negative MMR genes results in regulated genomic stability.

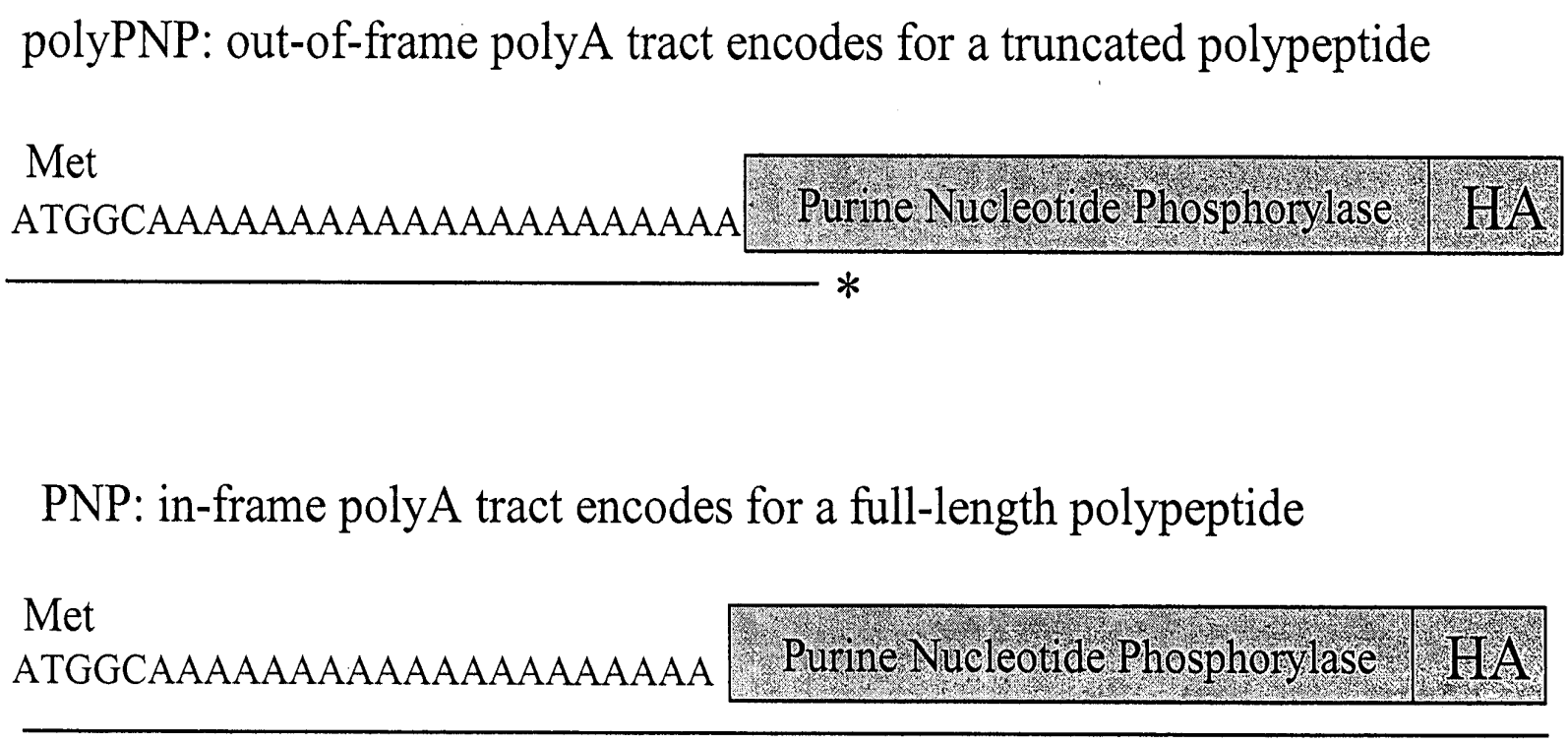


Figure 7: Schematic diagram of polyPNP and PNP genes. Both genes have a hemaglutinin (HA) tag at the C-terminus. Met indicates the initiating methionine codon.

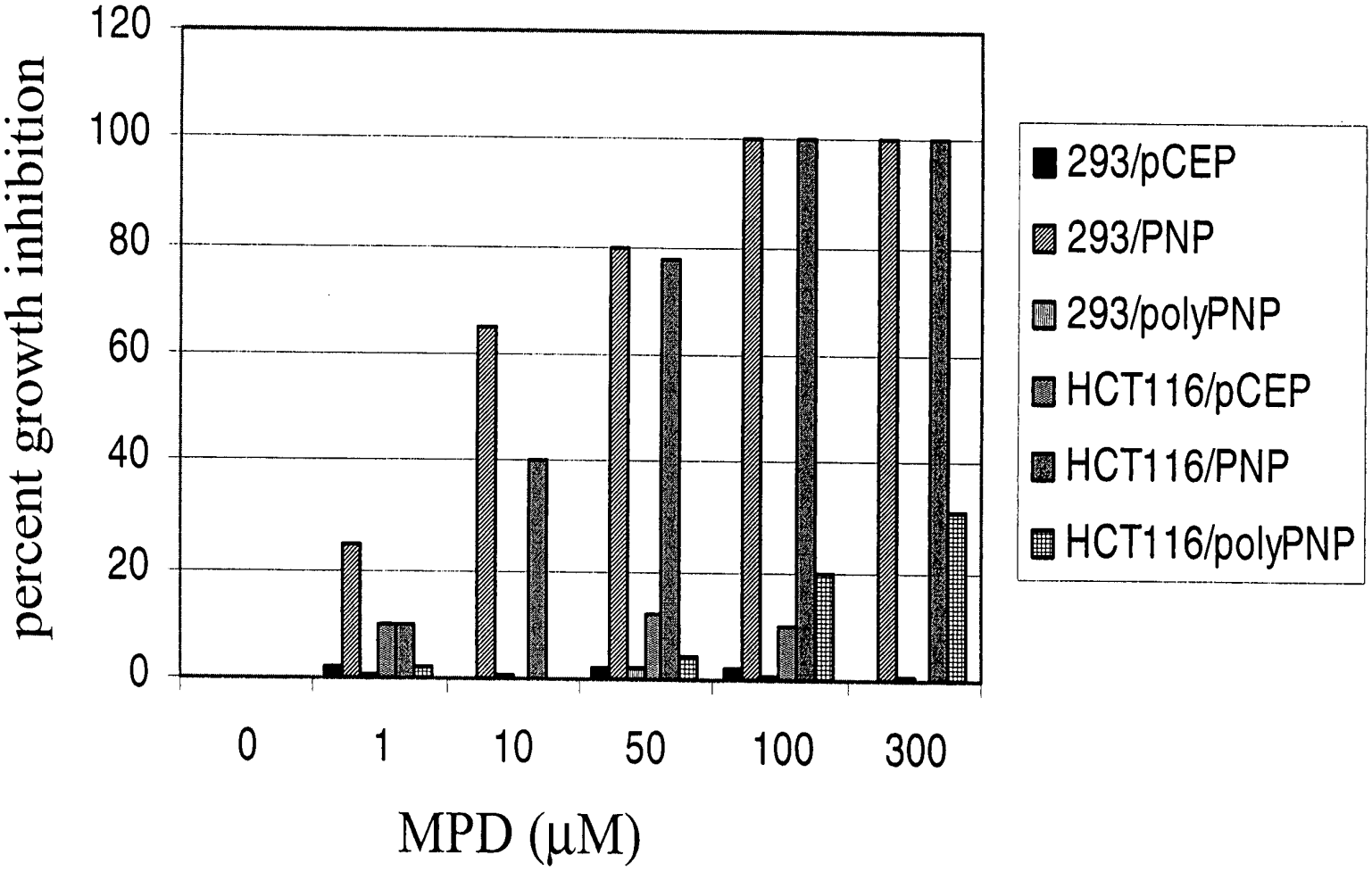


Figure 8A. Growth assay of HEK293 and HCT116 cells expressing PNP, polyPNP or pCEP empty vector. Cells were grown in varying concentrations of MPD for 10 days and counted for cell growth. The data shows that the polyPNP (hatched bars) gene is converted from an inactive allele to an active allele in HCT116 (MMR-deficient) cells but not in HEK293 (MMR proficient) cells

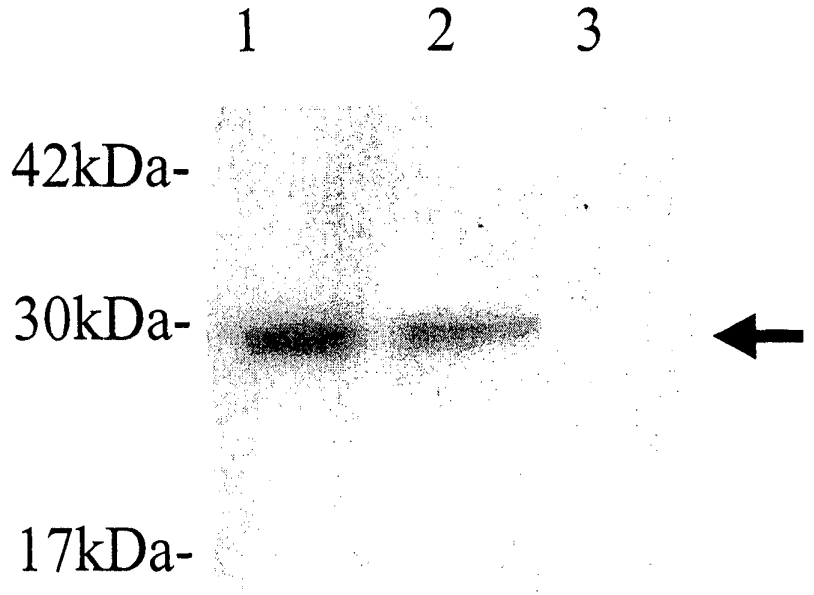


Figure 8B. Anti-hemaglutinin (HA) western blot of HCT116 cells expressing PNP (lane 1), polyPNP (lane 2), or pCEP (lane 3) empty vector. Arrow indicates a positive reacting polypeptide with the expected molecular weight. The data shows that the polyPNP gene structure is altered in vivo to produce an allele that originally contained an out-of-frame coding region to an allele containing an in-frame coding region

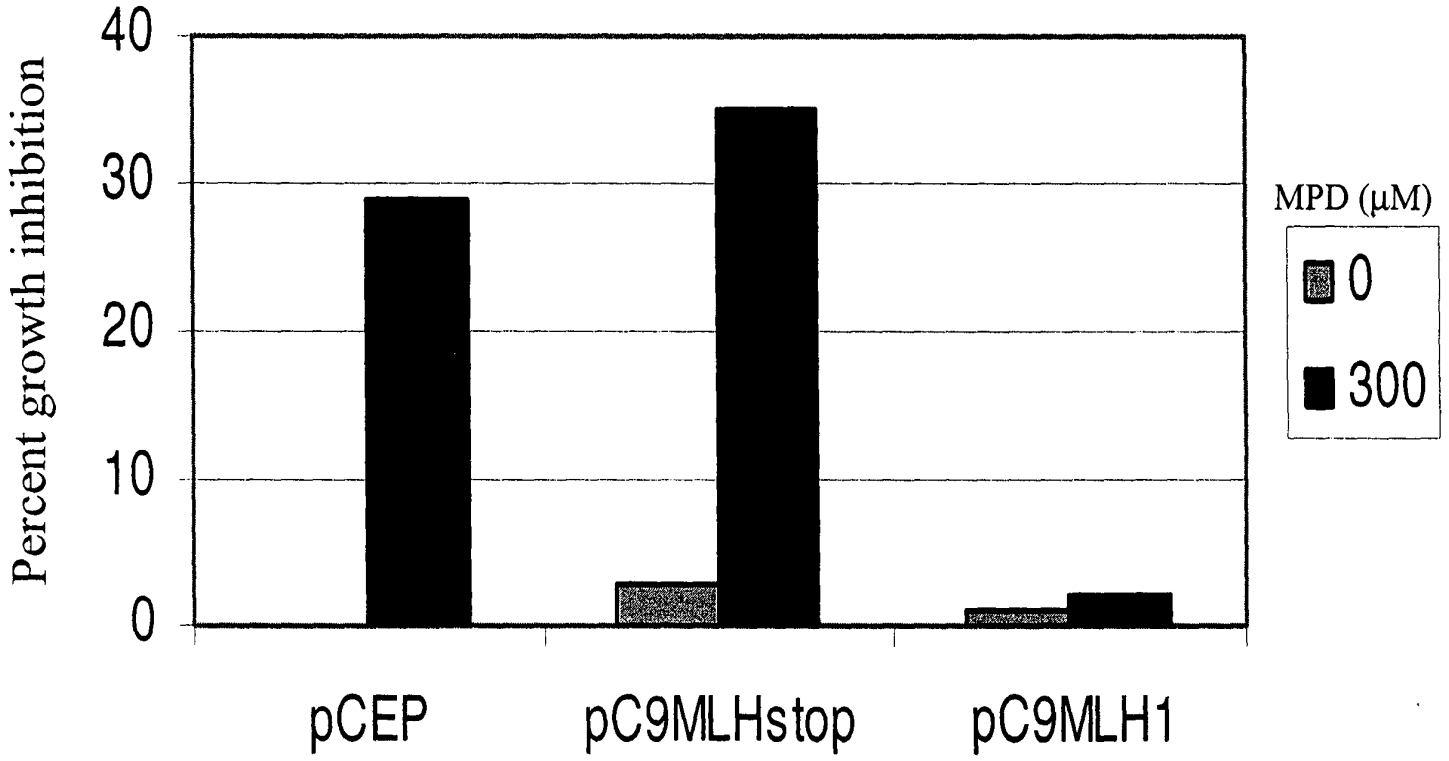


Figure 9. Growth inhibition assay of MMR-defective HCT116 cells expressing polyPNP plus pCEP9, MLHstop, or MLH1. Cells were grown in medium with or without 300μM MPD for 10 days and then counted for cell growth.
Shown is the mean of triplicate experiments

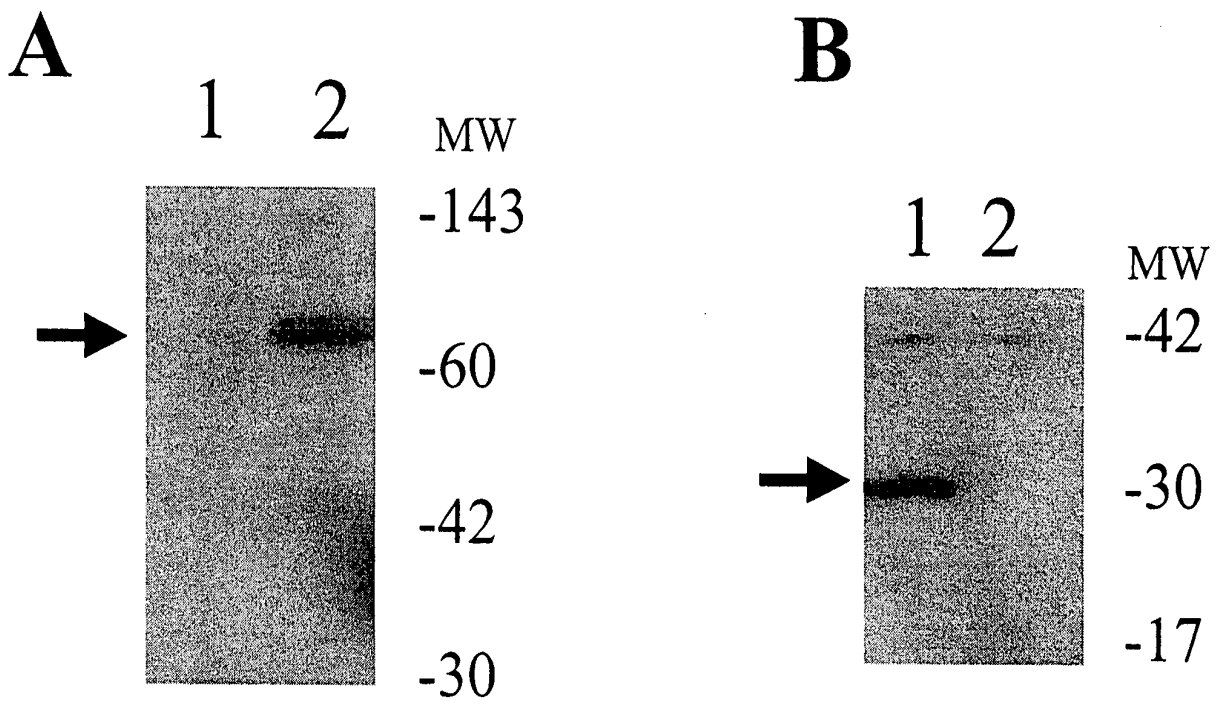


Figure 10. Western blots of HCT116 cells cotransfected with the polyPNP gene plus the MLHsto (lanes 1) or MLH wild type (lanes 2) expression vectors. (A) Cell lysates were probed for MLH1 using an MLH1-specific monoclonal antibody. The arrow indicates the correct molecular weight size for the full-length MLH1 protein. (B) Cell lysates were probed for HA using an HA-specific monoclonal antibody. The arrow indicates the correct molecular weight size for the full-length polyPNP protein. The numbers on the right side of each blot represent moleculr weight markers.